Supplemental Material
for

Roles of *fkbN* in Positive Regulation and *tcs7* in Negative Regulation of FK506 Biosynthesis in *Streptomyces* sp. KCTC 11604BP

Running Title: REGULATION OF FK506 BIOSYNTHESIS

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21   Table S1. Primers used in this study.

Primer	Sequence 5' to 3' (restriction site underline)	Restriction enzymes
PCR		
Tcs2_OF	GGACC<u>AGATCT</u>ATCTCGCACTACATTCGG	BglII
Tcs2_OR	GAATC<u>TCTAGA</u>CTAGCCCGGAGTCAG	XbaI
Tcs7_OF	GGACC<u>AGATCT</u>CGTCCGGTGATGGAATTCTG	BglII
Tcs7_OR	GAATC<u>TCTAGA</u>GGATTCTCAGTCCTGTGGGGCG	XbaI
FkbN_OF	AAATT<u>AGATCT</u>CATGGAGACGACGGCCGCGA	BglII
FkbN_OR	GTTAT<u>TCTAGA</u>CTACCCGCACCGGTCG	XbaI
Tcs7_LF	GGTC<u>TCTAGA</u>AGTTCGAACGCGAGCGT	XbaI
Tcs7_LR	GTT<u>GGATCC</u>GACGAAGTACTCCAGGGT	BamHI
Tcs7_RF	GTT<u>AGATCT</u>GACCCTCAACCCGCCC	BglII
Tcs7_RR	GCT<u>GATATC</u>ATCTCTAGAGTCCCCGCC	EcoRV
FkbN_LF	GTT<u>AAGCTT</u>TGCACAACCCGATCTGAT	HindIII
FkbN_LR	ATTATAT<u>CTAGA</u>CCGCCCCCGGCG	XbaI
FkbN_RF	ATTTAT<u>TCTAGA</u>CTCGCGGCCGTCGTC	XbaI
FkbN_RR	GCC<u>GAATTC</u>GTGCCGTCATTTGGTCG	EcoRI
RT-PCR		
TcsA_RTF	GGGAGCGCTTCTTCTACTCC	
TcsA_RTR	AGGGAGTCGACACCGAGAT	
TcsB_RTF	GGACTGTTACCCGACGACTG	
TcsB_RTR	CGATGACCCAGTCGACATC	
TcsC_RTF	CTACCGTCAGCTGGTGTCC	
TcsC_RTR	CATATGTGACCCGATGATGC	
TcsD_RTF	AGAGAGTGTGCGGATCGTCT	
TcsD_RTR	GTCGGAGAACGACACCTGAT	
Tcs1_RTF	ACACTGACGGAGACCGACAT	
Tcs1_RTR	GACCTCGAAGGACACGATTG	
Tcs2_RTF	AAAAGGTTGTCCTGGATTCG	
Tcs2_RTR	GAATACGAGGTGGGTCTCCA	
Tcs3_RTF	AACATGTTCACCGCCTATGG	
Tcs3_RTR	AGCAGGGTGTCGACGAGTT	

Tcs4_RTF	GGTCACCTTCCATCTCTCCA
Tcs4_RTR	GGCCGTATATCTCCTTGCTG
Tcs5_RTF	CTCGGTATGGGACATCGAAC
Tcs5_RTR	GTGACGTATCCGCCCTCTT
FkbG_RTF	TACGTCCGGAAGGTGTCACT
FkbG_RTR	TAGTAGGCCGGATAGCGTTC
FkbH_RTF	CTGGGATCTGGACAACACCT
FkbH_RTR	ATCCGGCCTGGTACATGAG
FkbI_RTF	CGCGAGACCTATCTGAAGGA
FkbI_RTR	GAACTGTTGCCGGCTCTTC
FkbK_RTF	CACTTCATGAACCCGTCGTA
FkbK_RTR	CACCGGAACACCTCTTCGTA
FkbL_RTF	GTCATCGAGTTCATGCCGTA
FkbL_RTR	GATGTGACGGTGCTCAGGA
FkbO_RTF	GGTATCACCTGCGTGTTCCT
FkbO_RTR	ACTCCTTCGATCTCCACGAG
FkbP_RTF	GCCTACGCGATCTACACCTC
FkbP_RTR	GGACCGTAGTGGTTGTGGAC
FkbD_RTF	ACAGCAGATGGTCGGCTACT
FkbD_RTR	GAGACCCTCGTTCATCGGTA
FkbM_RTF	GACGGTCTCGCACATCAAC
FkbM_RTR	GTCTCGATCTCTTCGCTCGT
FkbN_RTF	GAGCAGCCCCTTATGAACTG
FkbN_RTR	GAGGTGCAGCAGACAGAGC
FkbQ_RTF	GCTGTTCGGACACAGTATGG
FkbQ_RTR	ACTTGTTCCTGGTGCTCGAC
FkbR_RTF	TCAAGACCCTTCACCATCCT
FkbR_RTR	GTACCTGCACTCCCGGATAG
FkbA_RTF	GGTGAGAAGGTCCTGATCCA
FkbA_RTR	CTGCTCATCCAGCCGAAC

|	
FkbB_RTF	CATGGGCCTGAAACTGATG
FkbB_RTR	AGACGTTGTCGACCTGTGC
FkbC_RTF	GGAGGGATGGACGTTTCC
FkbC_RTR	AGGAGTCGGGTGATGATCTG

22
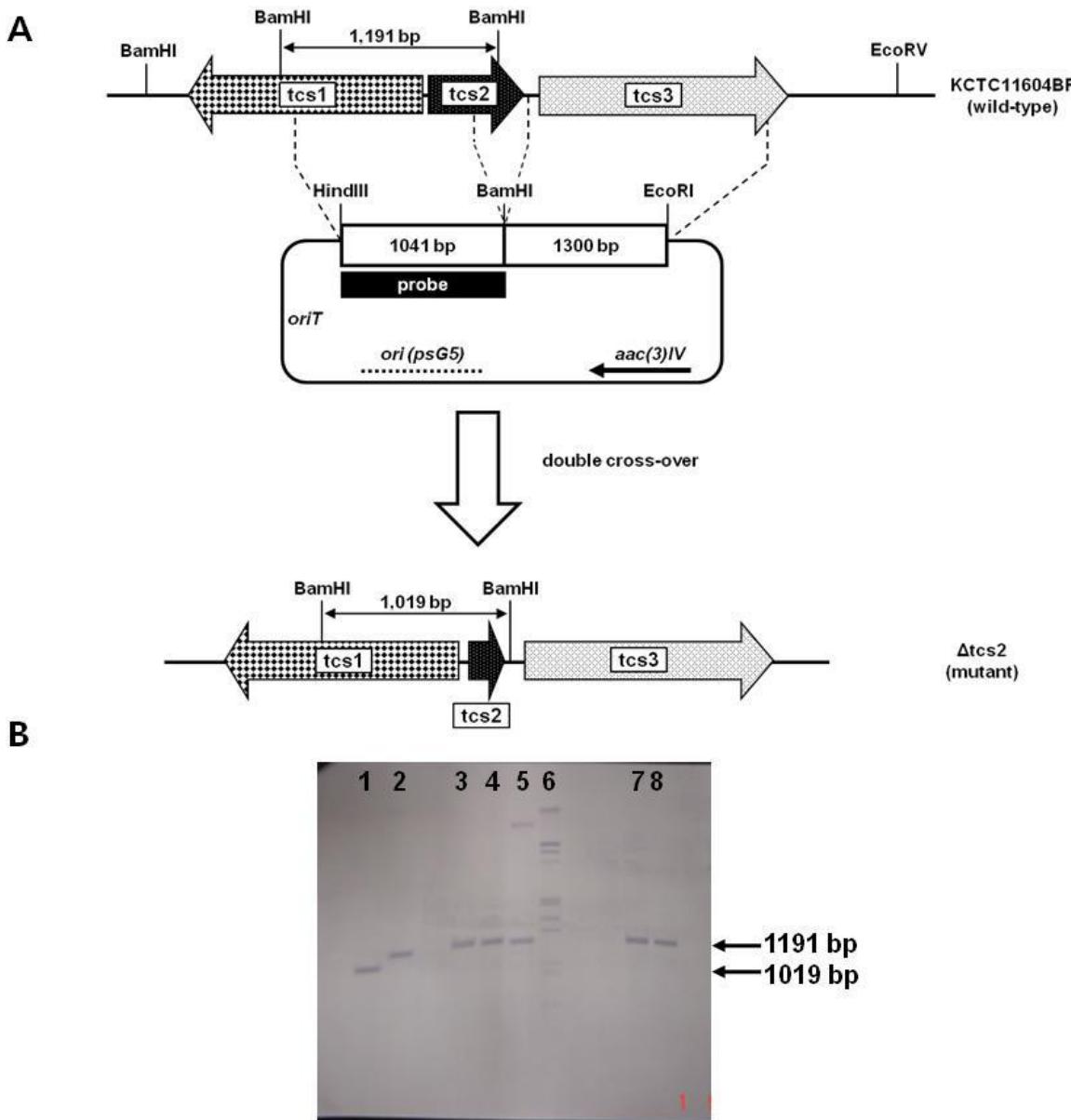
Fig. S1. Construction and verification of *tcs2* in-frame deletion in *Streptomyces* sp. KCTC 11604BP. (A) Schematic representation of *tcs2* in-frame deletion by homologous recombination. (B) Southern blot analysis. Genomic DNA restricted with BamHI from wild-type (lane 2), single-crossover mutant (lane 5), Δtcs2 mutant (lane 1), and wild-type revertant (lane 3, 4, 7, 8) strains. Molecular weight marker (lane 6). The indicated HindIII-BamHI fragment of 1041 bp was used as a probe (solid quadrangle).

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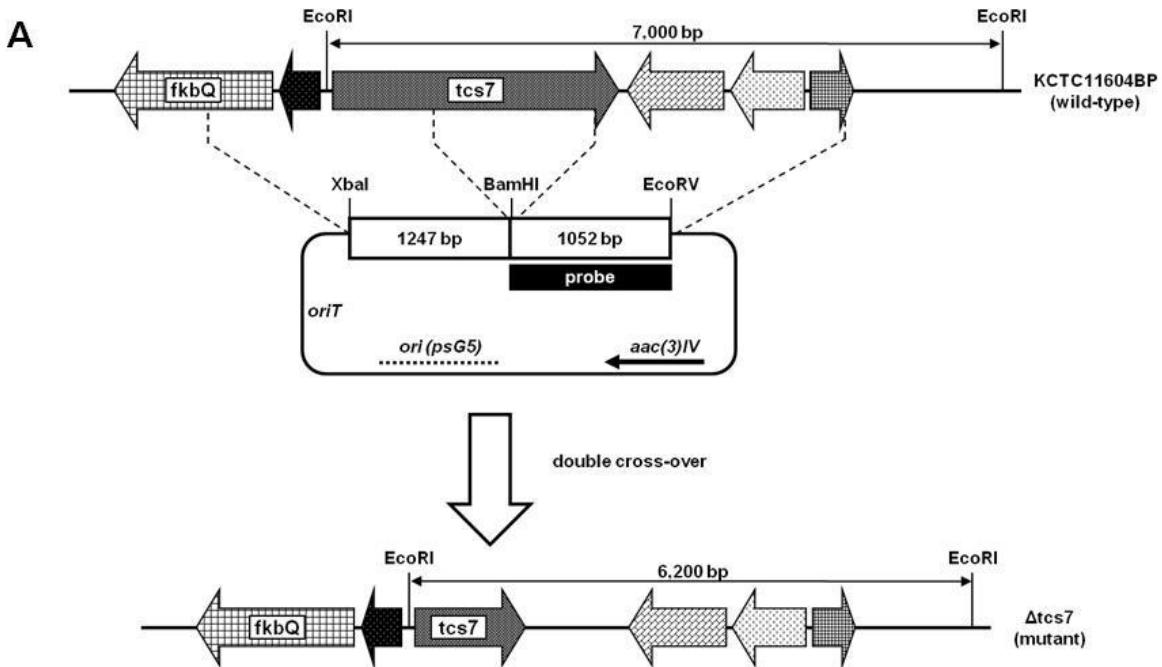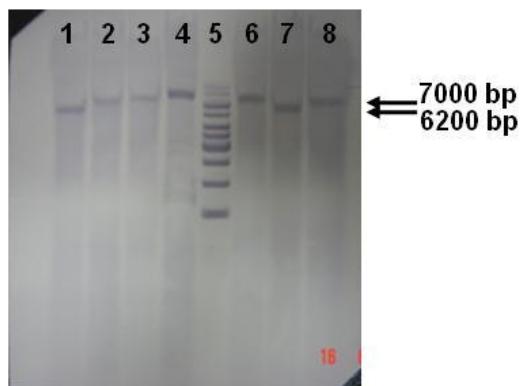
Fig. S2. Construction and verification of *tcs7* in-frame deletion in *Streptomyces* sp. KCTC 11604BP. (A) Schematic representation of *tcs7* in-frame deletion by homologous recombination. (B) Southern blot analysis. Genomic DNA restricted with EcoRI from wild-type (lane 2), single-crossover mutant (lane 4), Δtcs7 mutant (lane 1, 7), and wild-type revertant (lane 3, 6, 8) strains. Molecular weight marker (lane 5). The indicated BamHI-EcoRV fragment of 1052 bp was used as a probe (solid quadrangle).

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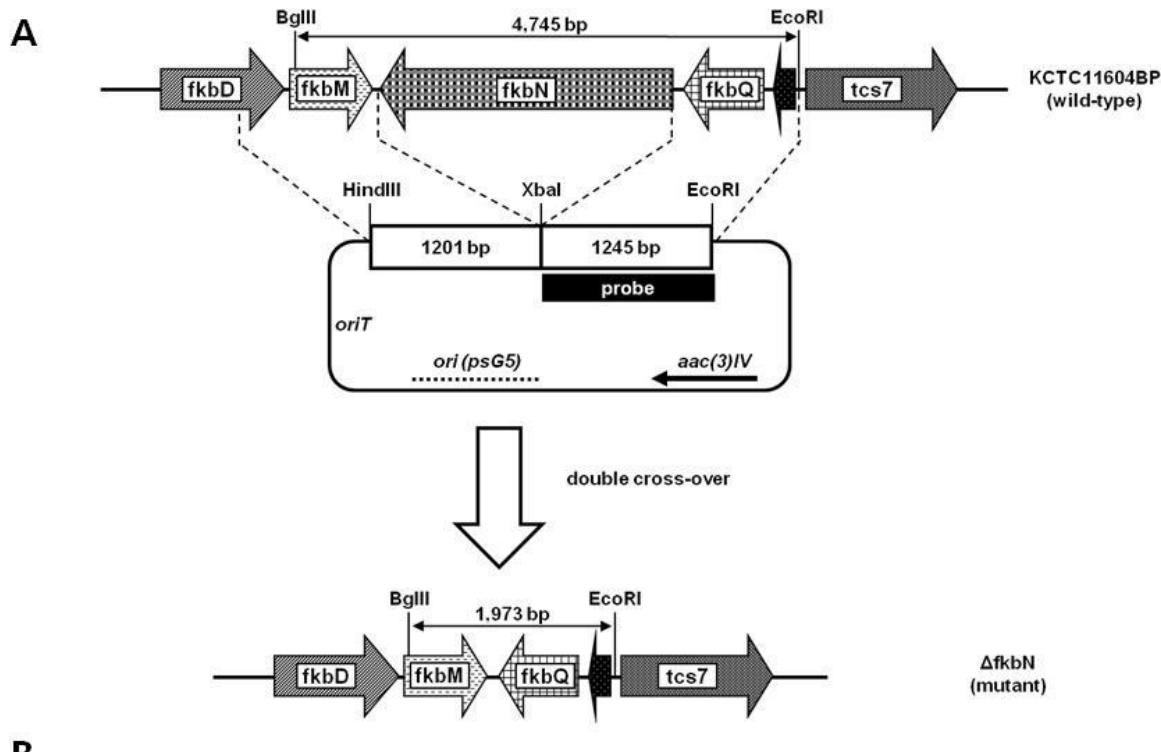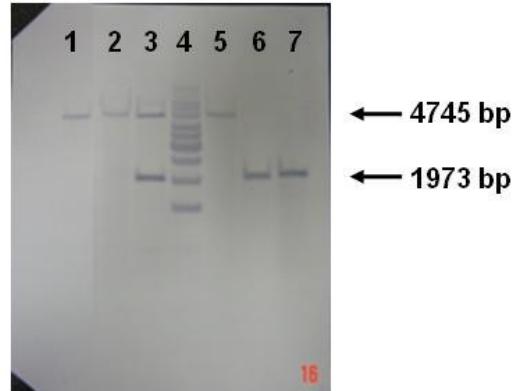
Fig. S3. Construction and verification of *fkbN* in-frame deletion in *Streptomyces* sp. KCTC 11604BP. (A) Schematic representation of *fkbN* in-frame deletion by homologous recombination. (B) Southern blot analysis. Genomic DNA restricted with BglII and EcoRI from wild-type (lane 1), single-crossover mutant (lane 3), ΔfkbN mutant (lane 6, 7), and wild-type revertant (lane 2, 5) strains. Molecular weight marker (lane 4). The indicated XbaI-EcoRI fragment of 1245 bp was used as a probe (solid quadrangle).